

## VOLTAMMETRIC DETERMINATION OF TRACE ELEMENTS (Cu, Pb, Zn) IN PELOID-BASED PHARMACEUTICALS

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**Abstract:** The research on the potential health risk posed to consumers by toxic elements that can be found in peloids is still lacking. Moreover, in Polish law no clinical or pharmacological tests are required to identify healing properties of peloids. The objective of this work was to determine some mineral content in selected peloids used in medical treatment. Anodic stripping voltammetry with differential pulse step was used for zinc, copper and lead determination. Decomposition of organic matrix was conducted by a simple wet digestion procedure using acid digestion vessel. Obtained results showed that proposed methods were suitable for the determination of investigated metallic elements. Lead content varied between 0.18 mg/kg (in Maść Borowinowa) and reached up to 15.5 mg/kg of dry weight for Chokrak peloid. Zinc content ranged from 0.64 to 66.87 mg/kg and copper content was between 0.57 and 7.50 mg/kg. The proposed method was validated, the recovery for peloid samples were 94 – 102%; 92 – 97%; 96 – 106% for copper, zinc and lead, respectively.

**Keywords:** trace element, muds, peloids, medical muds, therapeutic mud

Peloid is described as a matured mud or muddy dispersion with healing and/or cosmetic properties, composed of a complex mixture of fine-grained natural materials of geologic and/or biologic origins, mineral water or sea water, and common organic compounds of biological metabolic activity (1). The peloids also named as therapeutic muds, thermal muds, clay or ancient peat are known from prehistoric times and were used for healing purposes by Egyptians and Romans (2, 3). Currently, peloids are used in cosmetics and medical treatment in spa resorts. The application of peloids – called pelotherapy is becoming increasingly popular (4). The use of healing muds in pharmaceutical formulations as gastrointestinal protectors, oral laxatives, anti-diarrheas, dermatological protectors and in esthetic medicine, became more common during last few years due to increasing interest in natural medicine (5, 6). While being selected on empirical bases, all natural materials are not free of possible side-effects (7). Various components of mud, particularly fulvic acid, trace elements may be absorbed through the skin or any skin abrasions, cuts, or other

breaches in the integrity of the skin (8-13). Furthermore, after applying the mud directly on the body when it dries, released particles may be inhaled immediately, or they may be incorporated into house dust and enter the respiratory tract at a later time. Accidental mud swallowing by children or a transfer of the material from their hands to their mouths should also be considered. However, there has been no assessment of the potential health risk to consumers posed by toxic elements possibly present in the peloid itself and their toxicity are still lacking (6, 14). In the U.S., the Food and Drug Administration has limited authority over the cosmetics industry and rarely does any routine testing for toxic metals in products used for cosmetic purposes (15, 16). However, FDA regulations state that heavy metal concentrations in cosmetic products should not exceed 10 ppm in the case of Pb. According to European directives, lead is not allowed in cosmetic materials, including clays and peats for pelotherapy (85/391/CEE, 86/179/CEE and 86/199/CEE) (7). Potential toxicity associated with elemental composition of cosmetics is well documented in e.g., eye

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highlighters (17, 18) muds and clays (6, 10, 19) and other products (20, 21). Moreover, Polish Ministry Regulation in this case defines that peloids should be physico-chemically and microbiologically tested to determine their healing properties, but no clinical or pharmacological tests are required (Dz. U. 2006 Nr 80 No. 565).

Available databases show that spectroscopic methods (ICP-MS, ICP-AES, AAS, XRF) were used for determination of copper, zinc and lead in the majority of available research works (6, 22, 23). All the methods described above, are not free from interferences. The direct determination of metals in samples with high organic matter content by atomic absorption spectrometry is not always possible due to matrix composition. In that case liquid-liquid extraction, flow injection system and solid phase preconcentration techniques were applied (24, 25). The sequential voltammetric procedure allowing determination of zinc, copper and lead was rarely used in biological and environmental samples. Therefore, the application of simple electroanalytical methods should improve analytical performance. The aim of this research was to determine content of zinc, copper and lead in peloids and its products by anodic stripping voltammetry.

## MATERIAL AND METHODS

### Material

Artificial clays (peloids) were purchased in drugstore or directly in spa resort. All raw materials were conditioned in room temperature before use. Pasta borowinowa lecznicza – Biochem®, Borowinowa Kostka Iwonicka® from Iwonicz Spa (Poland), Maść borowinowa, Sulphur Zdrój® (Busko Zdrój, Poland), Lecznicza pasta borowinowa®, Kamień Pomorski Spa (Poland) were purchased in drugstore. Chokrak peloid sample was collected from its environment in the Chokrak lake area (Ukraine) in April and May, 2013.

### Apparatus

A Multipurpose Electrochemical Analyser M161 with a M164 electrode stand (both mtm-anko,

Poland) were used for all voltammetric measurements. The standard three-electrode cell consisted of a controlled growth mercury drop electrode (CGMDE) as a working electrode, Ag/AgCl in 3 M KCl with a double junction filled with 3 M KCl (Mineral, Poland) as reference and platinum wire as an auxiliary electrode. Voltammograms were recorded, interpreted and stored by EAGRAPH 6.0 (mtm-anko, Poland) software.

### Reagents

A standard solutions of  $\text{Zn}(\text{NO}_3)_2$ ,  $\text{Cu}(\text{NO}_3)_2$ , and  $\text{Pb}(\text{NO}_3)_2$  were prepared by proper dilution of 1 g/L stock standard solution (OUM, Łódź, Poland). Electrolyte used as ionic medium was prepared by dissolving  $\text{KNO}_3$  (Merck, Suprapur®). For digestion procedures  $\text{HNO}_3$  (Merck, Suprapur®) and 30%  $\text{H}_2\text{O}_2$  (Chemman, Poland) was used. All the solutions were prepared with double-distilled water from quartz distiller (SZ-97A, Chemland, Poland) and all other reagents were of analytical grade.

### Sample preparation

Samples were homogenized in agate mortar and then dried at over 70°C for 4 hours. Approximately 250 – 500 mg of sample material was accurately weighed and inserted in a Teflon® container of an acid digestion vessel (4748, Parr Instruments Co., USA). Next, the sample was treated with 5 – 6 mL of nitric acid and 1 mL of perhydrol, tightly sealed and was placed in drying oven (Binder, Germany). The digestion of the sample was carried 24 h in 160°C and after it cooled to the room temperature the vessel was unsealed and the sample was quantitatively transferred to an evaporation dish. The digested sample was placed at the heated plate for evaporation and removing the nitrates. The sample solutions were then cooled to room temperature, transferred quantitatively into volumetric flasks (10 mL) and filled up to the mark with double distilled water. All the procedures were carried out in triplicate for each sample.

### Analytical procedure

The voltammetric procedure for determination of copper, zinc and lead level was in accordance

Table 1. Analytical parameters of voltammetric analysis of selected trace elements.

Element	$E_{\text{acc}}$ [mV]	$t_{\text{acc}}$ [s]	Method	dE [mV]	Working electrode	Medium
Zn(II)	-1200	20	DP ASV	-20	CGMDE	0.2 M $\text{KNO}_3$
Cu(II)	-400	40	DP ASV	-20	CGMDE	0.04 M $\text{KNO}_3$
Pb(II)	-600	40	DP ASV	-20	CGMDE	0.04 M $\text{KNO}_3$

$E_{\text{acc}}$  - accumulation potential,  $t_{\text{acc}}$  - accumulation time, dE - pulse amplitude.

Table 2. Trace element and water content of investigated peloid products.

Product name	Trace element content (mg/kg d.w.)			
	Zn(II)	Cu(II)	Pb(II)	Water content [%]
Pasta borowinowa lecznicza - Biochem®	17.6 ± 1.5	1.6 ± 0.2	11.6 ± 3.2	92.31
Borowinowa Kostka Iwonicka® from Iwonicz Spa (Poland)	0.8 ± 0.2	0.7 ± 0.1	0.55 ± 0.17	80.26
Peloid from Chokrak lake (Ukraine)	66.9 ± 4.7	4.6 ± 0.4	15.5 ± 3.4	52.27
Maść borowinowa. Sulphur Zdrój® (Busko Zdrój. Poland)	1.0 ± 0.1	0.6 ± 0.1	0.18 ± 0.05	-*
Lecznicza pasta borowinowa® Kamień Pomorski Spa (Poland)	20.5 ± 7	7.5 ± 1.2	9.6 ± 1.0	80.67

\*40 g water extract of peloid/100 g ointment.

with a method that was previously used (26). Before each measurement the solution in the voltammetric cell was deaerated by high purity argon for 5 min and then, after redirecting the argon flow over the solution surface, measurements were performed. Zinc, copper, lead were determined using differential pulse anodic stripping voltammetry with a controlled growth mercury drop electrode (CGMDE) with a differential pulse stripping step (27). The measurements were performed according to the standard addition method, voltammograms corresponding to individual additions were registered three times

All experiments were carried out at room temperature. All instrumental parameters of performed measurements were arranged in Table 1.

### RESULTS AND DISCUSSION

The proposed voltammetric method using CGMDE after digestion procedure allows determination of zinc (20 s deposition time) with LOD = 0.49 µg/L and LOQ = 1.49 µg/L. The slope for regression line is  $-2.809 \pm 0.036$  (nA/µg/L) with a correlation coefficients  $r = 0.9989$ . The recovery of selected peloid samples ranged from 92 to 97% for zinc. The proposed method also allows the determination of copper (40 s deposition time) with LOD = 1.71 µg/L and LOQ = 5.15 µg/L. The slope for regression line is  $-2.399 \pm 0.019$  (nA/µg/L) with correlation coefficients  $r = 0.9967$ . The recovery of selected samples ranged from 94 to 102% for copper. Furthermore, the same method also allows determination of lead (40 s deposition time) with LOD = 0.15 µg/L and LOQ = 0.47 µg/L. The slope for regression line is  $-1.949 \pm 0.036$  (nA/µg/L) with correlation coefficients  $r = 0.9995$ . The recovery of

selected peloids ranged from 96 to 106% for lead. For cadmium only LOD and LOQ were estimated at the level 0.09 µg/L and 0.27 µg/L, respectively. The investigated samples showed cadmium level below detection limit and no interpretable signal indicated cadmium presence. During zinc and copper analysis a 1000-fold excess of Fe (III), and 100-fold excess of Pb (II), Cd (II), Mn (II) did not interfere. The surface-active compounds are usually a source of strong interferences in voltammetric methods and should be thoroughly destroyed by digestion prior to analysis.

Zinc showed the highest concentration levels in natural peloid samples, whereas the lowest level values were observed for Pb (II) (Table 2). The highest zinc content was determined in natural samples from Chokrak lake 66.9 mg/kg and the lowest was in pharmaceutical product Borowinowa Kostka lecznicza 0.8 mg/kg. The highest value of copper concentration was determined also in Chokrak lake peloid (4.6 mg/kg) and the lowest was in pharmaceutical ointment Maść borowinowa (0.6 mg/kg). The highest lead contamination was determined in peloid from Chokrak lake 15.5 mg/kg with the smallest moisture. Basing on obtained results positive correlations were observed between the investigated trace elements contents: very high between zinc and lead ( $r = 0.86$ ) and high in pairs Cu-Pb ( $r = 0.61$ ) and Zn-Cu ( $r = 0.53$ ).

The investigated samples were different in origin and a direct comparison is difficult. Among them the Chokrak mud was interesting because it provided a reliable test for the effectiveness of analytical method. Additionally, there are no available data of voltammetric analysis of trace element in peloids. When comparing the results obtained in this study, to the ones from spectroscopic analysis it can

be observed that both methods give the same range of concentrations. Chokrak mud zinc and lead content were in the range showed by other works conducted over Portuguese mud where approximately 50 to 150 mg/kg of zinc and 5 to 38 mg/kg of lead were found (6). Moreover, Spanish peloids showed similar zinc and lead concentration with a range between 33.1 - 89.8 mg/kg and 10.9 - 37.5 mg/kg, respectively (23). Peculiarly, according to prior research some peloids can show zinc content up to 160 mg/kg with elevated levels of lead and copper. In comparison, copper level in Chokrak peloid was two times lower than the lowest of previously reported content and in average five to ten times lower than the highest value showed in earlier research (23). However, the distribution of element content was similar showing the following order: Zn > Cu > Pb.

The two types of paste, first from Spa resort and second from market, had similar zinc and lead contents but differed in copper. Correlation between water content and element content were quite low ( $r = 0.42$ ) mostly due to lead and copper contents. For zinc and water content solely, correlation coefficient showed high value ( $r = 0.86$ ) but was statistically insignificant. This fact indicates that all the peloids pastes have similar zinc concentration taking into account their water content. According to regulations, the investigated samples can be considered free from heavy metal contamination excluding the peloid from Chokrak lake and Pasta borowinowa lecznicza (Biochem). In this case the lead content was above 10 mg/kg and exceeded the value approved by Polish Pharmacopoeia. However, the limits were exceeded only when calculated for dry sample mass. When water is taken into consideration the contents of lead in the peloid from Chokrak lake and Pasta borowinowa lecznicza – Biochem were 7.39 mg/kg and 0.89 mg/kg, respectively. Moreover, the peloid paste or peloid mud in medical treatment are used in hydrated form directly on the body, so the exceeded limits should not be considered in this case.

## CONCLUSIONS

It is necessary to expand study on more samples, but at Polish market there is only a limited number of peloid-based products. However, this study gives an overview on products that are available in Poland and a single, less known peloid from its natural source. The obtained results showed that voltammetric methods used in this study were suitable for determination of copper, zinc and lead both in peloids and derived products. The proposed method is characterized by

low instrumental and single analysis costs, ten times lower than the usually applied methods, achieving comparable results with spectroscopic analysis. According to FDA and EC regulations, a trace element content study and analytical quantification for selected elements seem to be necessary, especially when concerning their life important functions and/or their toxicological hazard.

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